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Crocidura cossyrensis Contoli, 1989 (Mammalia, Soricidae): karyotype, biochemical genetics and hybridization experiments

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***Crocidura cossyrensis* Contoli, 1989 (Mammalia, Soricidae): karyotype, biochemical genetics and hybridization experiments. -** The shrew *Crocidura cossyrensis* Contoli, 1989 from Pantelleria (I), a Mediterranean island 100 km south of Sicily and 70 km west from Tunisia, was investigated in order to understand its origin and its relationship with *C. russula* from Tunisia, Morocco and Switzerland. With the exception of a single heterozygote centric fusion, *C. cossyrensis* had a karyotype identical with that of *C. russula* from Tunisia (2N = 42, NF = 70 to 72), but it differed from *C. russula* from Morocco and Switzerland (2N = 42, NF = 60). The former have 5-6 pairs of chromosomes with small arms that are acrocentric in the latter. Genetic comparisons with allozyme data revealed small genetic distance (0.04) between *C. cossyrensis* and *C. russula* from Tunisia. In contrast, this eastern clade (Tunisia and Pantelleria) is separated from the western clade (Switzerland and Morocco) by a genetic distance of 0.14. A hybridization experiment between shrews from Pantelleria and Switzerland lead rapidly to an F1 generation. From 12 F1 hybrids that were backcrossed, females reproduced normally, but none of the males did so. Concluding from the results, *C. cossyrensis* from Pantelleria and *C. russula* cf. *agilis* from Tunisia belong to the same taxon that may have reached the differentiation of a biological species within the *C. russula* group. More geographic samples are needed to determine the definitive taxonomic positions of these shrews.

Keywords: Soricidae - *Crocidura* - Pantelleria - Tunisia - phylogeography - chromosomes - hybrids.

INTRODUCTION

The Mediterranean island of Pantelleria (Italy) is situated 100 km southwest of Sicily and about 70 km east of the Tunisian coast. It is of volcanic origin; the oldest volcanic event is dated 220'000 years B.P., the most recent eruption occurred 8'000 years B.P. (Civetta *et al.*, 1984). As on most Mediterranean islands, a small mammal

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community is present, composed of typical elements of anthropic origin such as *Mus domesticus, Rattus* sp., *Apodemus sylvaticus* and two white toothed shrews, *Suncus etruscus* and a species of the genus *Crocidura*. First discovered as subfossil remains, the material was tentatively assigned by Felten & Storch (1970) to *Crocidura russula*. More recently, a live specimen was trapped (Contoli & Amori, 1986) and provisionally identified as *C. russula*. Later on, Contoli (in Contoli *et al.,* 1989) suggested in an infrapaginal remark specific rank for this island population and named it *C. cossyrensis*. Finally, an identification key and other morphological traits were presented by Contoli (1990), who suggests to verify this taxonomic conclusion by other techniques.

Such a verification was carried out in 1990/1991 and preliminary results (Vogel *et al.,* 1992: Abstract in *Israel Journal of Zoology* 38: 424) suggested a close relationship of *C. cossyrensis* with *C. russula* from Tunisia. A morphological study (Sarà & Vogel, 1996) revealed a discontinuity between *C. russula* from Tunisia and Morocco, confirmed by a *cytochrome b* study (Vogel *et al.*, 2003). Finally, a new investigation of mtDNA (Lo Brutto *et al.*, 2004) confirms a close relationship between the shrews from Pantelleria and Tunisia, contrasting with *C. russula* from continental Europe. We present here evidences from karyology, an allozymic study and breeding experiments, which shed new light on shrews from Pantelleria compared to shrews from Europe, Tunisia and Morocco.

MATERIAL AND METHODS

The shrews from Pantelleria were collected by M.S., P.V. and Laura Zanca between 21 and 24 March 1990. We captured 1 *Sucus etruscus* and 8 *Crocidura cossyrensis*. Five were trapped at four places along the road from Siba (at 300 m) to Montagna Grande (800 m) and three around the lake Bagno dell'Acqua. For a comparison, 2 *C. russula*, captured by M.S. in Ain Draham (Tunisia) in July 1990 were included in this study. Moreover, for the hybridisation experiment, some *C. russula* from Switzerland were used. These shrews are shown in Figure 1.

The chromosomes were prepared from bone marrow with the air drying technique (Baker *et al.,* 1982), stained with Giemsa and prepared by G-banding (*C. cossyrensis*: 4 individuals: IZEA-3834, IZEA-3853, IZEA-4184, IZEA-4222; *C. russula* from Tunisia 2 individuals: IZEA-3897, IZEA-3898; hybrids 3 individuals: IZEA-4296, IZEA-4634, IZEA-4704). For a comparison, chromosomes of *C. russula* from Switzerland were reanalysed (IZEA-840, IZEA-1004, IZEA-1181, IZEA-2227).

The allozyme analysis was performed from liver, heart and kidney tissues using vertical starch gel electrophoresis following methods described in Maddalena (1990). It was based on three *C. cossyrensis* captured at different places in the field (IZEA-3834, IZEA-3853 and IZEA3895), the two *C. russula* from Tunisia (IZEA-3897, IZEA-3898) compared with data formerly published (Maddalena, 1990) concerning 5 *C. russula russula*, from Morges (Switzerland) and 10 *C. russula* from Oukaimeden and Imlil (Morocco). The amount of genetic divergence between populations was estimated by using the index of standard genetic distance (D) proposed by Nei (1978).

From the captured *C. cossyrensis*, six were used as founders for a breeding colony established at IZEA in Lausanne. The shrews were kept in the same conditions

FIG. 1

A. *Crocidura russula* cf. *agilis* from Tunisia; B. *Crocidura cossyrensis* from Pantelleria; C. *Crocidura russula* from Switzerland. The pictures were taken in a standard cage (scale bar: 1 cm).

as *C. russula* described by Genoud & Vogel (1990). During summer 1990, three litters of *C. cossyrensis* with a total of 8 young were produced. In order to assess reproductive compatibility, a program of hybridisation was set up that started also in summer 1990 by forming mixed pairs of *C. cossyrensis* with *C. russula* from Switzerland, resulting

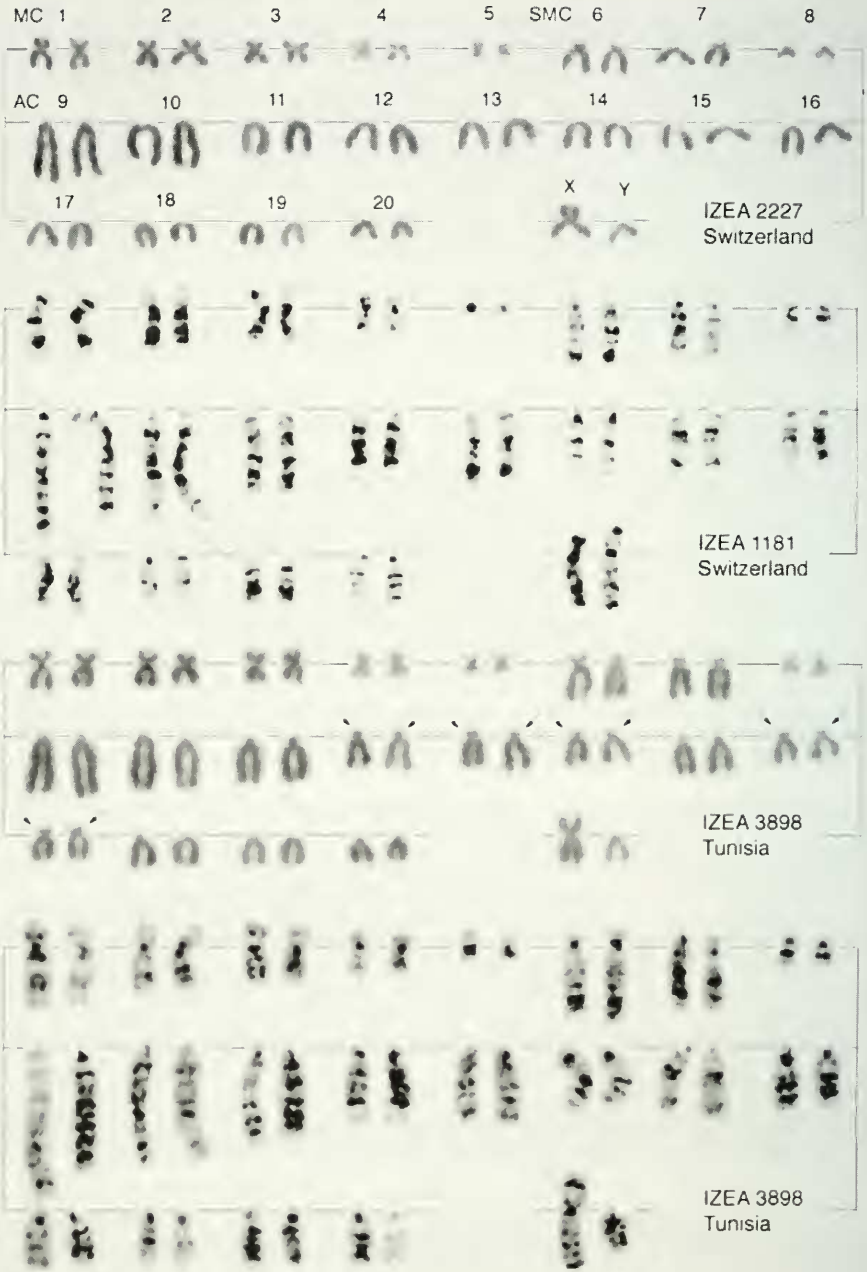

FIG. 2

Giemsa stained and G-banded karyotype of *Crocidura russula* from Switzerland (from Maddalena, 1990, but modified in the interpretation) and *C. russula* from Tunisia. MC = metacentrics, SMC = submetacentrics, AC = acrocentrics; arrows indicate supplementary chromosome arms.

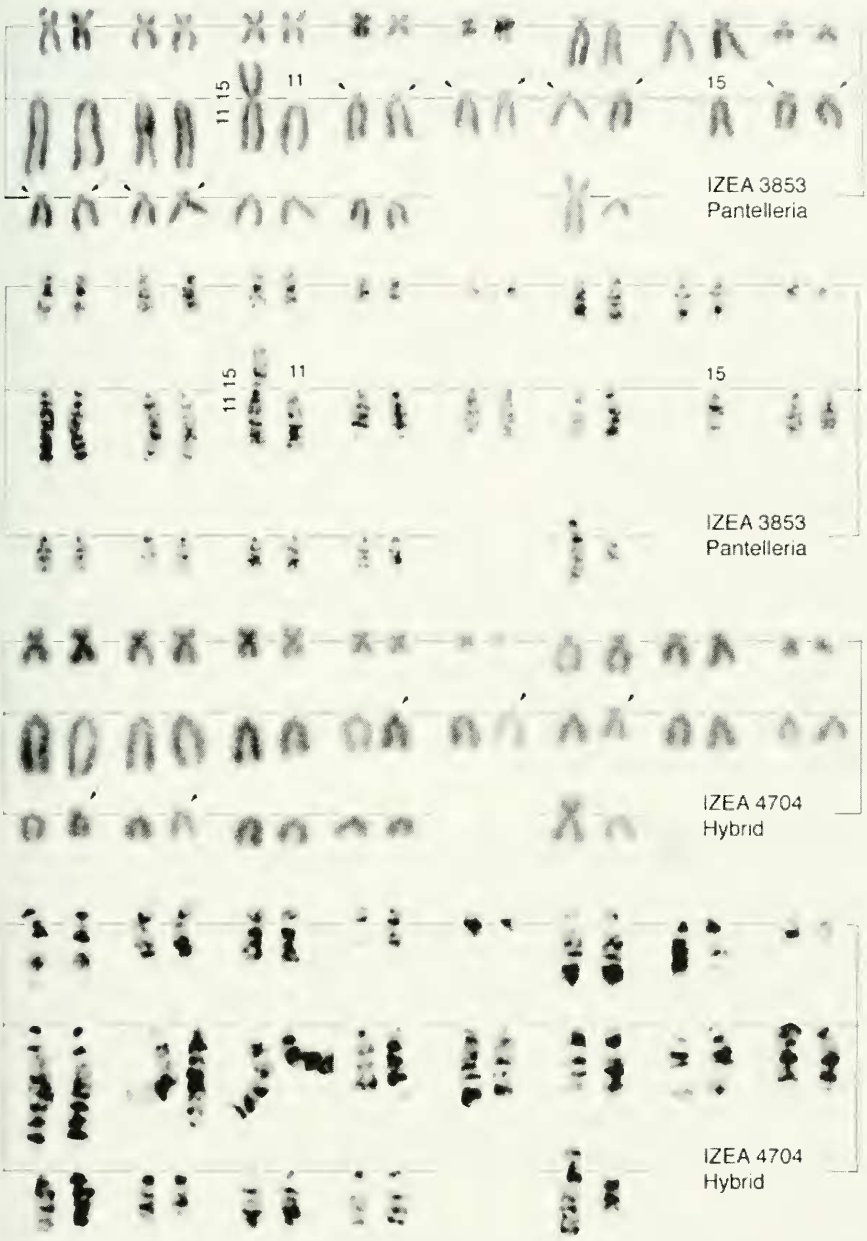

FIG. 3

Giemsa stained and G-banded karyotype of *Crocidura cossyrensis* from Pantelleria (IZEA 5853) with 2N = 41; and of a Hybride (IZEA 4704). Arrows indicate supplementary chromosome arms.

in 5 litters with a total of 13 F1 hybrids. In 1991, after a period of winter inactivity, 12 hybrids (5 females and 7 males) were associated with *C. cossyrensis* (5 females, 1 male) and 7 *C. russula* from Switzerland (3 females, 4 males). The pairs were formed during 3 to 6 weeks. Such a back-cross experiment should show if the fertility of hybrids is normal.

RESULTS

The karyotype

The classical Giemsa stained preparation revealed for *C. cossyrensis* as well as for *C. russula* from Tunisia a basic karyotype of 2N = 42, similar to that of *C. russula* from Switzerland (Fig. 2 – 3), but not identical. Five pairs were metacentric, 8 or 9 were submetacentric and 6 or 7 pairs were acrocentric, resulting in a NF = 70 – 72 (definition of FN see Reumer and Meylan, 1986) instead of a NF = 60. The X-chromosome was metacentric and the Y acrocentric. In G-banding, the small arms did not appear and revealed the homology between the chromosomes of each individual, independent of its origin (Pantelleria, Tunisia and Switzerland). One male *C. cossyrensis* (IZEA-3853), the young of a pregnant captured female (IZEA-4184), showed a karyotype of 2N = 41, clearly heterozygous with one Robertsonian fusion between chromosome 11 and 15 (Fig. 3). Theoretically, the mother (IZEA-4184) could have been 2N = 42, 41 or 40; her control revealed the same heterozygosity (2N = 41).

In the analysis of G-banding preparations of F1 hybrids (*C. cossyrensis* x *C. russula*), homologous chromosomes were easily recognised, whereas the association of the Giemsa stained chromosomes with the small arms in heterozygote state was a bit more problematic (Fig. 3).

Allozyme comparison

Three specimens from Pantelleria were compared by electrophoresis to *C. russula* from Morocco, Switzerland and Tunisia. Of the 32 loci analyzed, 25 were monomorphic (Adh, Ak, Alb, Ada, Ck-1, Ck-2, Est-1, Est-2, Got-1, Got-2, a-Gpd, Hbb, Hk, Hpd, Ipo-7, Ipo-8, Lap, Ldh-1, Ldh-2, Mdh-1, Mdh-2, Pa, 6-Pgd, Pgi and Sdh) and seven polymorphic (Table 1). The shrews of Pantelleria can be differentiated by the allele Mod^{-109} that is not present in the other populations. The G-6-pd^{114} allele is common for the shrew of Pantelleria and Tunisia. Nei's 1978 values of genetic distance (D) were calculated among populations (Table 2). They were low between Pantelleria and Tunisia (0.04) and Morocco and Switzerland (0.03), but rather high between Tunisia and Morocco (0.14) and Pantelleria and Morocco (0.18).

Hybridisation experiments

For the fertility experiment, 12 of the F1 hybrids (*C. cossyrensis* x *C. russula* from Switzerland) were used for back-crossing with six pure *C. cossyrensis* born in summer 1990 and seven *C. russula* from Switzerland. Moreover, we tried also the combination between F1 hybrids. The results are shown in Table 3. Many of the pairwise associations were unsuccessful. This may happen even between fertile individuals (H-22 x Cr-34 or H-22 x Cr-35). After numerous combinations, more than 50% of all

TABLE 1. Alleles (and allelic frequencies) for polymorphic allozyme loci of *Crocidura cossyrensis* from Pantelleria and *C. russula* from Tunisia, Switzerland and Morocco.

	Morocco n = 10	Switzerland n = 5	Pantelleria n = 3	Tunisia n = 2
G-6-pd	110 (.90)	110 (.80)	114 (1)	114 (.50)
	112 (.10)	112 (.20)		90 (.50)
Idh-1	150 (.80)	150 (1)	150 (1)	150 (1)
100 (.20)				
Idh-2	-100 (.30)	-100 (.90)	-100 (1)	-100 (1)
	-75 (.70)	-75 (.10)		
Ipo-9	92 (1)	92 (1)	93 (1)	93 (1)
Mod	-121 (1)	-121 (1)	-109 (1)	-121 (1)
Mpi	137 (.30)	137 (.20)	137 (1)	137 (1)
	100 (.70)	100 (.80)		
Pgm	80 (.10)		80 (.67)	80 (1)
	100 (.90)	100 (1)	20 (.33)	

TABLE 2. Matrix of the standard genetic distances (Nei, 1978) calculated between four populations of *Crocidura russula* and *C. cossyrensis* analysed by protein electrophoresis.

	Morocco	Switzerland	Pantelleria
Switzerland	0.031		
Pantelleria	0.179	0.145	
Tunisia	0.137	0.105	0.039

TABLE 3. Reproductive success between *Crocidura cossyrensis* (Cc) of the first generation rized in laboratory, *C. russula* (Cr) and hybrids (H). In the cells are given either the litter size or the infertile association (-).

sex			m	m	m	m	m	m	m	m	m	m	m	m
	type		Cc	H	H	H	H	H	H	H	Cr	Cr	Cr	Cr
		N°	27	14	17	18	19	20	21	24	32	34	35	36
f	Cc	9	-	-		-			-					
f	Cc	11			-	-	-			-	1			
f	Cc	12		-		-	-		-			2+4		
f	Cc	13		-			-		-				1	
f	Cc	28		-	-	-		-						-
f	H	15	-			-						6+5		
f	H	22			-					-	4+4	-	-	
f	H	23										-	-	-
f	H	25	-	-					-		5+4			
f	H	26					-	-					-	-
f	Cr	31	-		-			-			-			
f	Cr	33		-	-			-	-			2		
f	Cr	37			-			-		-				3+5

categories combined with males and females of *C. russula* produced litters, e.g. 60% of the female F1 hybrids. However, no female became pregnant from a pairing with an F1 hybrid male. This result suggests reduced fertility in hybrid males, but not in hybrid females.

DISCUSSION

Based on morphological features, Contoli (in Contoli *et al.*, 1989) interpreted the shrew of Pantelleria as an endemic island species. Our results show that this shrew is very close to *C. russula* from Tunisia, but differs from *C. russula* of Europe and Morocco.

In Europe, until now, each *Crocidura* species was considered to have a specific karyotype (Vogel *et al.*, 1990). According to the synthesis of Reumer & Meylan, (1986), *C. russula* from Europe is characterised by 2N = 42, NF = 60. Exactly the same was found in *C. russula* from Morocco (Hutterer *et al.*, 1987). The here presented results in regard to *C. cossyrensis* from Pantelleria and *C. russula* from Tunisia showed the same diploid number of chromosomes (2N = 42) and the same banding pattern permitting to identify the same chromosomes. However, five to six pairs of normally acrocentric chromosomes have small arms, leading to a submetacentric morphology. Such small arms have been occasionally observed in European populations, e.g. by Schmid (1968) in one pair, and by Catalan (1984) in five chromosome pairs in one out of four karyotyped shrews from the island Ibiza, Spain (*Crocidura russula ibizensis*). In contrast to these reports, the shrews from Pantelleria and Tunisia show always a high number of submetacentric chromosomes, revealing without doubt a close phylogenetic relationship.

This result is confirmed by the allozyme analysis showing a close genetic relationship between the Tunisian and the Pantellerian populations. The genetic distances are typical for intraspecific populations and are of the same order as the genetic distance between the populations from Morocco and Europe, the latter having been derived from the former probably at the end of the last glaciation (Catzeflis *et al.*, 1985; Vogel & Maddalena, 1987).

Nei's mean genetic distance D = 0.14 between the two clades is rather high for an intraspecific differentiation, but of the same level as between some populations in *C. suaveolens* (Catzeflis *et al.*, 1985). These data from nuclear genes confirm results based on the cytochrome *b* gene (Vogel *et al.*, 2003). In those analyses, *C. russula* as well as *C. suaveolens* seem to present species groups rather than single species. The split of the *C. russula* group into an eastern and western clade, was also evidenced by Lo Brutto *et al.* (2004) based on SSR and 12S-rRNA genes.

Finally, the results of our hybridization experiment indicate a clear but somewhat disrupted relationship between the European *C. russula* and the shrew from Pantelleria: An F1 generation and backcrosses from hybrid females were easily obtained, but hybrid males did not reproduce, suggesting at least partial sterility, in agreement with Haldane's rule.

From these results, the following taxonomic conclusion may be outlined:

i) The Tunisian population, characterized by numerous pairs of submetacentric chromosomes and also by two "private alleles" (shared with *C. cossyrensis*) should be separated from *C. russula yebalensis* Cabrera, 1913 from Morocco. Sarà & Vogel (1996) assigned the Tunisian population provisionally to *C. russula* cf *agilis* Levaillant, 1867, which was described from Algeria (Ellerman & Morrison-Scott, 1951). According to morphometric analyses (Sarà & Vogel, 1996), the geographic border between the two taxa is situated in eastern Algeria, as shown by a stepped cline.

ii) The shrew population on Pantelleria derived from a Tunisian population, most probably during historical time. The colonisation followed thus the classical model of postglacial human introduction as shown for the hedgehog *Erinaceus algirus* of Malta (Malec & Storch, 1972), *C. suaveolens* of Crete (Vogel *et al.*, 1986) and Corsica (Catalan, 1984; Maddalena, 1990) and *C. russula* of the Canary Islands (Vogel *et al.*, 2003).

iii) The clade including *C. cossyrensis* and *C. russula* from Tunisia may have reached the level of a biological species within the *C. russula* group. Samples from the whole geographic area of the group are needed to determine the definitive taxonomical position of these shrews.

A final remark concerns the shrews from Ibiza studied by Catalan *et al.* (1988). This island population compared to other European *C. russula* showed with a genetic distances of Nei of 0.1 the most isolated position, due to two specific alleles (in Aa-1^{120} and Np-R^{132}), while other alleles where shared with populations of Spain. As explanation, the authors supposed an African origin. The occurrence of karyotypes with and without supplementary small chromosome arms suggests a mixed origin, from continental Europe and from the eastern North African clade (*C. r.* cf *agilis*).

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